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ADDENDUM

Auxin action in developing maize coleoptiles: challenges and open questions

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ABSTRACT
The year 2020 marks the 150th anniversary of the elucidation of the process of plant organ growth at the cellular level by Julius Sachs (1870). In this Addendum to a Review Article in *Molecular Plant*, we describe this fundamental discovery and argue that the etiolated grass coleoptile still represents the system of choice for the experimental analysis of auxin (indole-3-acetic acid, IAA)-action. With reference to the phenomenon of 'tissue tension', we discuss the acid-growth hypotheses of IAA-induced wall loosening and the process of vacuolar expansion, respectively. IAA-mediated elongation appears to be independent of wall acidification, and may be regulated via the secretion of glycoproteins into the outer epidermal wall, whereby turgor (and tissue) pressure provides the 'driving force' for growth. As predicted by the "acid growth-hypothesis", the fungal phytotoxin Fusicoccin (Fc) induces organ elongation via the rapid secretion of protons. We conclude that "cell elongation" can only be understood at the level of the entire organ that displays biomechanical features not established by single cells. This systems-level approach can be traced back to the work of Sachs (1870).

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Introduction

Last year, Matthes et al. (2019) published a Review Article[1] in the journal *Molecular Plant* entitled "Auxin EvoDevo: Conservation and diversification of genes regulating auxin biosynthesis, transport, and signaling". In this comprehensive summary, the authors focused on a phylogenetic analysis of auxin (indole-3-acetic acid, IAA)-related genes in the crop species maize (*Zea mays*) and rice (*Oryza sativa*). However, the physiological response to IAA at the organ level was not addressed. Since Matthes et al. (2019)[1] extensively discussed "current challenges and future directions for auxin research", we want to supplement their agenda, with reference to monocots/IAA action and a focus on auxin physiology in maize at the organ level. In addition, we commemorate the 150th anniversary of the first description of the process of cell elongation by the German plant physiologist Julius Sachs (1832–1897), who introduced maize seedlings for the experimental study of plant growth and development.

The etiolated seedling as model system for cell biology

One hundred and fifty years ago, Sachs (1870) published the second, much improved edition of his seminal *Lehrbuch der Botanik* (*Textbook of Botany*), wherein seedling development in "Turkish Wheat" (maize) was described in detail (Figure 1a). In addition, the process of organ growth was depicted at the cellular level for the first time in the history of plant science.[2] In a famous scheme, Sachs (1870) illustrated non-vacuolated, apical cells of a meristem, as well as the cells in the elongation- and maturation zone of a developing root.[3] In this woodcut (Figure 1b), the scientist/artist Sachs illustrated his discovery that cell expansion is based on vacuolization, water-uptake, and turgor-driven elongation of meristematic cells. This process leads to an increase in size, accompanied by the differentiation of the expanded cells (i.e., the development of root hairs, which elongate via tip growth). In this context, Sachs[2] described the phenomenon of 'tissue tension', i.e., the fact that, in stems, the epidermal wall layer(s) are under tension and therefore restrict cell growth at the level of the entire organ. In addition, he discovered that stem elongation is promoted by darkening of the juvenile plants (etiolation).

In an earlier publication, Sachs (1862) had analyzed the relationship between the acidity of different regions of expanding roots and their growth-status.[4] He observed that the apical (meristematic) region is basic, the elongation zone "almost neutral", and the apical portion "decidedly acid". With these remarks,[4,5] he was an early pioneer in a research area that, one century later, led to the formulation of the so-called "acid growth-hypothesis" of cell elongation.[6,7]

In a series of articles, auxin-induced coleoptile elongation was analyzed in detail, with the aim to elucidate the mode of action of this phytohormone.[8–10] In these papers, the authors commented on the relationship between hormone action and acid growth. In a recent *Review Article*, Arsuffi and Braybrook[11] summarized their view on this topic, with which we disagree in a number of respects.

In the next sections, we present our perspective of auxin action on cell elongation in bamboos and grass coleoptiles, taking a "systems approach" into account[12] that can be traced back to the seminal work of Sachs.[2,3,5]

Auxin and shoot elongation: the bamboo-enigma

It has long been known that the rate of growth, measured in millimeters per hour (mm/h), differs widely between species,

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Dedicated to the memory of Winslow R. Briggs (1928–2019)

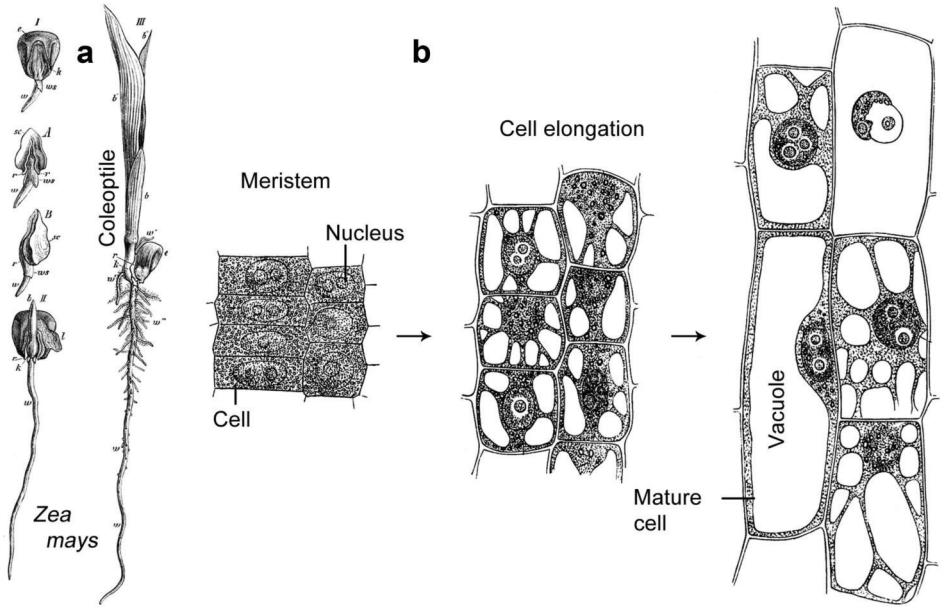

Figure 1. Development of a maize (*Zea mays*) seedling (a). Cell elongation in a growing root (b). Meristematic activity, followed by vacuolization, and the concomitant anisotropic expansion of the cells. In this classical woodcut, Julius Sachs illustrated a fundamental feature of plant cells, i.e., their ability to form large, water-filled vacuoles leading to turgor-driven plastic deformation (enlargement) of the cells (adapted from drawings of Julius Sachs.[2]

organs, and environmental conditions (i.e., the availability of water, temperature, moisture content of the air, etc.[2,5]). Using intact, etiolated grass seedlings that were raised in closed plastic boxes under optimal environmental conditions (moist vermiculite, 25°C), the following maximum growth rates during the linear phase of organ expansion were measured in darkness: maize (*Zea mays*): 0.4 mm/h; oats (*Avena sativa*): 0.9 mm/h; wheat (*Triticum aestivum*): 1.2 mm/h; rye (*Secale cereale*): 1.0 mm/h, and barley (*Hordeum vulgare*): 1.4 mm/h.[13] In etiolated seedlings of sunflower (*Helianthus annuus*), a maximum rate of hypocotyl growth of ca. 1.2 mm/h has been recorded (unpublished results). In the model plant *Arabidopsis thaliana*, the etiolated hypocotyl grows at a maximum rate of ca. 1.0 mm/h.[14] so that it is obvious that this value represents an average rate of shoot growth for many model species investigated in the laboratory. In all of these studies, seedlings were raised under controlled conditions, and it has been documented that IAA, combined with other phytohormones, is the key factor in the regulation of organ expansion.

In a recent monograph entitled *Bamboo: The Plant and Its Uses*, Liese and Köhl (2015)[15] have summarized evidence showing that in members of the subfamily Bamusoideae (family *Poaceae*), the only grass lineage that diversifies in tropical forests, much higher elongation rates than those mentioned above were recorded. For instance, in shoots of the bamboo species Moso (*Phyllostachys edulis*), a maximum rate of growth of more than 1 m per night has been measured (ca. 100 mm/h). However, the question whether or not auxin is involved in the regulation of rapid internode elongation in bamboos has been unclear. In a comprehensive analysis, Wei et al. (2018)[16] have shown that in rapidly growing shoots (vs. a slowly expanding mutant) numerous genes are up-regulated that are related to IAA-biosynthesis, signaling and response.

Based on Wei et al.'s detailed data set we may conclude that IAA represents the "universal" key hormone in the regulation of cell elongation throughout the plant kingdom, from grass seedlings (Figure 1a) via *Arabidopsis* to the most rapidly growing plants on Earth, the bamboos.[15,16] However, it should be mentioned that Wei et al. (2018) did not quantify the IAA content and its correlation to the rate of organ growth. Apparently, this problem has not yet been resolved.

Experimental analysis of coleoptile growth

Figure 2 (inset) shows a 4-d-old etiolated maize seedling. Sections, cut from the sub-apical region of intact coleoptiles that grow at a maximum rate, cease to elongate when incubated on water, i.e., in the absence of the phytohormone. As a result of the addition of IAA (usually applied 1 h after cutting), the coleoptile sections rapidly resume elongation over the subsequent 6–8 h. The data set also shows that, when the hormone is provided one, two or 4 h later, the sensitivity to IAA (at an applied optimal concentration of 10 μM) remains largely constant (Figure 2). This result indicates that, at least over the first 4 h after cutting, the experimental system is stable, i.e., no negative effects of the destructive process of cutting the sections are apparent (for instance, a wounding reaction of the cells that negatively impacts IAA-responsiveness after longer time periods).

In a classical study with the miniaturized pressure-probe, it has been documented that cell turgor of the thin-walled, extensible inner tissues of maize coleoptile (i.e., mesophyll, vascular bundles and inner epidermis) is 0.58 ± 0.01 MPa.[17] This "tissue pressure" acts on the thick, growth-controlling outer epidermal wall (OEW) of the coleoptile, and maintains this organ wall under longitudinal tension. This finding can be traced back to the work of Sachs (1870),[2] who described the phenomenon of

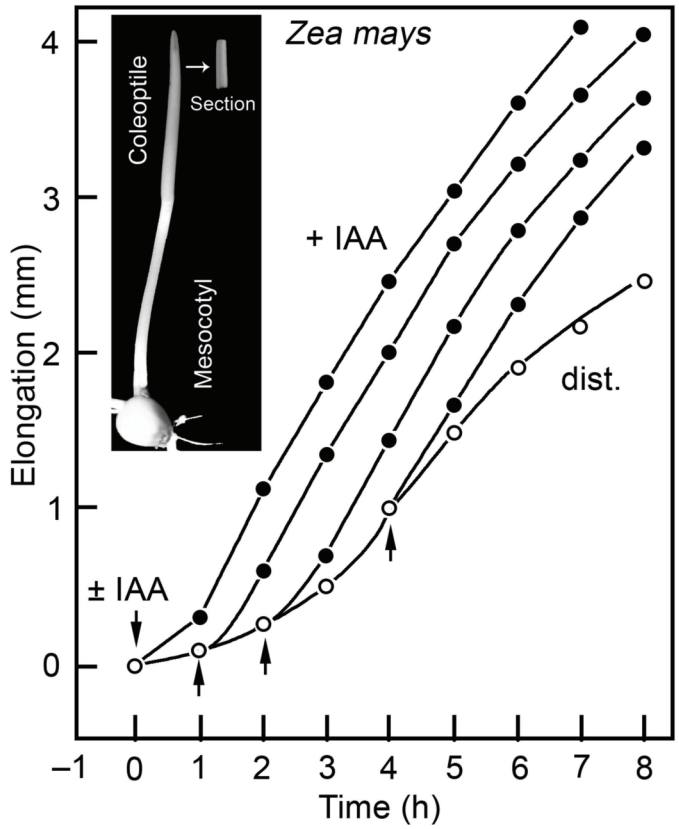

Figure 2. The dark-grown maize coleoptile (*Zea mays*) as model organism for the experimental analysis of auxin (IAA)-mediated cell elongation. A 4-d-old etiolated maize seedling (inset) is composed of the primary root (not shown) and the shoot, consisting of the mesocotyl and the coleoptile. Sections, 10 mm in length, were cut from the sub-apical region and incubated either in water (dist.) or an IAA-solution (10 µM). After 1 h of preincubation on water (depletion of endogenous auxin), IAA was added at time zero; in three other experiments, auxin was applied after 2, 3 or 5 h (original data).

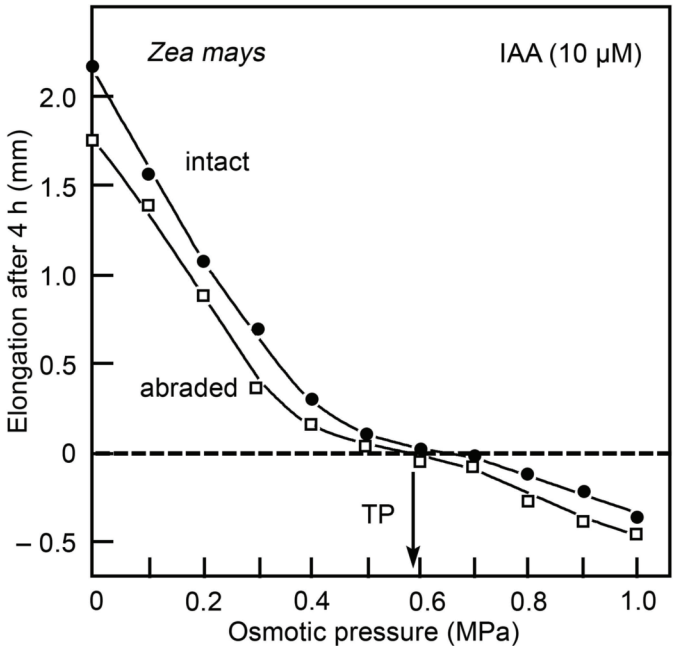

Figure 3. Biophysical basis of organ elongation in dark-grown maize (*Zea mays*) coleoptile sections that were either left intact or abraded with moist polishing cloth (opening of the apoplastic space). The sections were incubated in the presence of IAA (10 µM) as shown in Figure 3 (addition of auxin 1 h post-cutting), either in the absence or presence of an osmoticum (polyethylene-glycol) of defined osmotic pressures (0–1.0 MPa). Elongation after 4 h was plotted against the osmotic pressure of the solution. In the intact system, tissue pressure (TP) (arrow) was similar to cell turgor, as measured with a pressure probe (ca. 0.58 MPa) (original data).

'tissue tension' in some detail (see Figure 4a, which depicts a classical "peeling"-experiment).

Figure 3 shows that, using a growth recorder-assisted bioassay, the average turgor value of 0.58 MPa, measured with a pressure probe, can be approximately verified. Sections were incubated in water or osmotic solutions of 0.1 to 1.0 MPa (polyethylene-glycol), and elongation after 4 h was recorded. Both intact and abraded sections respond similarly. Hence, at an externally applied osmotic pressure of ca. 0.6 MPa (intact) and ca. 0.56 MPa (abraded), respectively, zero elongation is reached (= osmotic equilibrium). At higher osmotic pressures, shrinkage of the coleoptile sections occurs.

This finding indicates that the turgor (and tissue)-pressure of the internal cells is ca. 0.6 MPa (intact organ). Based on these (and other) biophysical studies, a model of the grass coleoptile has been deduced that is shown in Figure 4b. According to this analysis/interpretation of stem-like organs, the outer epidermal wall (OEW) is maintained under longitudinal tension by the turgid inner tissues. As a result, the initiation of rapid cell elongation, induced by IAA (Figure 2), is achieved via the loosening of the expansion-limiting peripheral organ wall. This biophysical model is also known under the name "epidermis-in-control-theory" and has been corroborated by numerous data-sets, obtained on a variety of turgid, axial plant organs.[17]

The enigmatic role of the epidermis

In his textbook of 1870, Sachs,[2] with reference to pioneering work of his colleague Wilhelm Hofmeister (1824–1877), described the phenomenon of tissue tension, i.e., the fact that when the epidermal cell layer from a coleoptile or stem is carefully peeled off, it will contract by ca. 10%. On the other hand, peeled sections of coleoptiles or stems, from which the epidermis was removed by more than 95% will, upon transfer to water, rapidly elongate (Figure 4a). Based on such experiments, combined with the so-called split-section-essay, it has long been known that the primary target tissue for IAA is the epidermal cell layer.[17] However, detailed quantitative studies on the biomechanical role and cellulose-architecture of the OEW revealed that the peripheral "wall-sheath" is under longitudinal tension, due to the action of the turgor pressure of the soft, thin-walled internal tissues. Accordingly, in the coleoptile, turgor (i.e. tissue)-pressure (P_V) and the longitudinal wall stress within the OEW (P_W) are counter-forces (Figure 4b). In stem-like organs, such as the sunflower hypocotyl, two sub-epidermal cell layers are also thickened; they display, like in maize-coleoptiles, a heliocoidal cellulose architecture, so that, in these cases, three peripheral walls are under tension. However, due to the lack of a neighboring cell, the OEW appears to bear by far the largest physical stress, and therefore must be interpreted as an "organ wall".[18–21]

Decades of auxin research with coleoptile sections has shown that this phytohormone rapidly causes the loosening (i.e. an enhancement in the plastic extensibility) of the OEW.

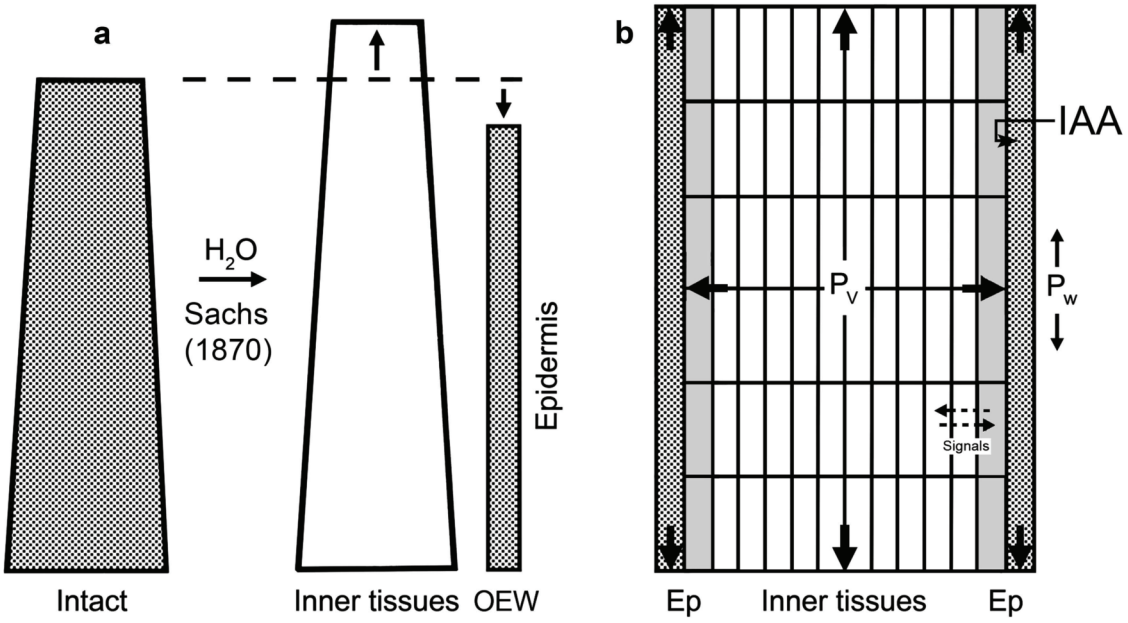

Figure 4. Schematic illustration of 'tissue tension' in the coleoptile of a grass seedling (or stem), as revealed experimentally by Julius Sachs.[2] (a). An intact segment, 1 to 2 cm in length, was peeled, and the resulting inner tissue cylinder (without the epidermis) placed on water (result: rapid turgor-driven elongation by ca. 10% within 1 h). The epidermal cell layer spontaneously contracts (–10%) due to the mechanical properties of the sturdy outer epidermal wall (OEW), which is maintained under tension in the intact organ. A biophysical model of the coleoptile (b) documents the phenomenon of 'tissue tension': the turgor pressure of the soft (extensible) internal cells (P_V) is borne by the peripheral organ wall (OEW) that is maintained under tension (wall-pressure, P_W). In addition, it is indicated that auxin (IAA) specifically acts in the epidermal cells, were it causes loosening of the OEW; as a result, turgor-driven organ elongation occurs (original scheme).

In contrast, wall plasticity of the thin inner cell layers remains high and largely unaffected by auxin.[18–21] Accordingly, the internal walls do not limit growth, at least over the first 8 h of auxin treatment in a growth-recorder (Figure 2).

How does auxin loosen the outer epidermal wall?

As mentioned in the Introduction, Sachs[2] was the first physiologist to study the relationship between tissue acidity and its corresponding physiological status (growing vs. mature). He discovered that there is a correlation between cell elongation and acidity of the cells (presumably the walls), at least with respect to roots. However, it was the American plant biochemist James F. Bonner (1910–1996) who discovered, in a landmark-paper of 1934, the "acid-growth-effect". With reference to earlier studies, Bonner (1934)[22] documented that, when coleoptile segments are incubated in acid solutions (pH ca. 4.0), they rapidly elongate over a period of 1 to 2 h, as if they would have been incubated in IAA. This acid-induced growth response was found to be significantly enhanced when the waxy cuticle of the epidermal cells was, at least in part, removed (abraded sections). As Figure 3 shows, abraded maize coleoptile sections are more sensitive toward osmotic solutions compared with the intact control, i.e., they shrink more rapidly. Hence, water (and acid) permeability is larger in abraded segments, compared to non-abraded control sections.

Nearly five decades ago, Cleland (1970)[23] and Hager et al. (1971)[24] independently proposed that auxin rapidly causes the initiation of cell elongation in excised segments via the secretion of protons, leading to the acidification of "the cell wall". Unfortunately, these pioneers of modern IAA-research did not refer to the work of Sachs (1870, 1887),[2,5] Bonner (1934),[22] and many others, who had clearly shown that, in coleoptiles and stems, the sturdy outer wall(s) are the growth-limiting structure(s). Accordingly, over subsequent decades, the role of the epidermal cell layer was largely ignored, until a major "*Zea mays* study" was published.[18]

In this comprehensive experimental analysis, it was shown that IAA causes the initiation of organ elongation via the loosening of the OEW; this physiological process was found to occur independent of IAA-mediated secretion of protons. A number of subsequent studies have shown that the "acid growth-hypothesis" applies to the fungal phytotoxin Fusicoccin (FC), but not to IAA. Since, in all of these numerous studies on auxin action, experiments using FC were included as positive controls, the conclusion that IAA causes wall-loosening of the OEW independent of its effect on proton secretion was inescapable.[6,7,10]

The acid balloon hypothesis

In a recent publication authored by Arsuffi and Braybrook,[11] the facts that (1) "acid-growth" is a real-world phenomenon and (2) the postulate of a causal relationship between IAA-induced wall-acidification and the initiation of wall expansion are mixed up. Hence, despite the fact that this article provides a solid overview of the history of "acid-growth-research", we think that the logic of some of their arguments is questionable. In addition, we disagree with Arsuffi and Braybrook's endorsement of the "acid balloon-theory" proposed 5 y ago by Dünser and Kleine-Vehn.[25] These authors speculate that IAA may act on the vacuolar membrane (tonoplast) by enhancing the uptake of solutes (see the scheme of Sachs 1870, Figure 1b). According to such a speculative scenario, IAA-induced coleoptile elongation should be caused by an enhancement in

turgor (and osmotic) pressure. However, numerous "auxin studies" have shown that, at least in grass coleoptiles and hypocotyl segments, no such "IAA-turgor-effect" exists. The cells of these organs behave analogous to an osmometer, i.e., turgor pressure slowly declines as the cells take up water and expand irreversibly.[17]

A biophysical model of auxin action in grass coleoptiles

With respect to the "acid balloon-hypothesis", we may conclude that plant organ growth can be compared with an automobile in action: Turgor pressure (i.e., the engine) provides the relatively constant 'driving force' for expansion, whereas the regulation of the rate of elongation (i.e., speed control) is achieved by means of a modulation of plastic extensibility of the peripheral wall (OEW). The question as to how IAA achieves its control over the "irreversible stretching-ability" of the OEW is one of the greatest enigmas in plant biology since the 1930s.[10–12,22–24] As mentioned above, IAA-induced wall acidification appears to be insufficient to promote cell elongation. As an alternative, a "cytological model" has been proposed and recently described in detail.[10] Numerous studies on the ultrastructure of the OEW (specifically, the periplasmic space), as well as proteomic analyses, have documented that IAA-mediated growth is accompanied by the occurrence of osmiophilic nanoparticles that are secreted via the Golgi-apparatus of the epidermal cells and fuse with the inner, expansion-limiting regions of the OEW. However, it is still not clear whether or not these osmiophilic deposits represent the enigmatic "wall-loosening factor" in the grass coleoptile with respect to the IAA-mediated growth.[26] More work is required to solve this open question.

Conclusions

In their Review Article on "Auxin Evo Devo", Matthes et al. (2019)[1] refer to "shoot development" and "organogenesis" in developing maize plants. Since the authors did not mention the biophysical basis of stem elongation, the aim of this Addendum was to provide information on this important topic. We want to stress that the biophysical model of stem growth depicted in Figure 4a,b can be traced back to the work of Sachs,[2,5] who described not only the processes of organ growth and cell enlargement (Figure 1a,b) but also the phenomenon of "tissue tension" and its bearing on the mechanism of stem elongation. Hence, a systems-level approach is required in future attempts to elucidate the enigmatic biochemical processes within the growth-controlling OEW, which are rapidly initiated in response to the application of auxin.

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Disclosure of Potential Conflicts of Interest

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